Both Horatio and Polonius: Innate Lymphoid Cells in Tissue Homeostasis and Repair

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ABSTRACT

Innate lymphoid cells (ILCs) have emerged as critical tissue-resident lymphocytes that coordinate responses to environmental stress and injury. Traditionally, their function was thought to mirror adaptive lymphocytes that respond to specific pathogens. However, recent work has uncovered a more central role for ILCs in maintaining homeostasis even in the absence of infection. ILCs are now better conceptualized as an environmental rheostat that helps maintain the local tissue setpoint during environmental challenge by integrating sensory stimuli to direct homeostatic barrier and repair programs. In this article, we trace the developmental origins of ILCs, relate how ILCs sense danger signals, and describe their subsequent engagement of appropriate repair responses using a general paradigm of ILCs functioning as central controllers in tissue circuits. We propose that these interactions form the basis for how ILC subsets maintain organ function and organismal homeostasis, with important implications for human health. *ImmunoHorizons*, 2023, 7: 729–736.

INTRODUCTION

In Shakespeare's *Hamlet*, Horatio and Polonius both serve as advisers to Danish royalty, the former in service to Prince Hamlet, who seeks retributive justice for his father's demise, and the latter allied with King Claudius, who usurps the throne after murdering his brother by poison. Yet, despite their similar positions, Horatio's and Polonius's interpretations of environmental cues, subsequent counsel to their benefactors, and the consequences of their actions stand in contrast to one another. Polonius is conniving and impertinent, misinterpreting the actions of the court despite his employment of spies and subterfuge. His schemes are the catalyst that drives much of the excess violence and pathologic injury that plagues the play's final act. In contrast, Horatio serves as a humanistic, educated friend and adviser to Hamlet, an impartial observer who confirms the presence of King Hamlet's ghost, remains privy to the prince's feigned court madness, and emblemizes loyalty and faithful observation. At the conclusion, Polonius is the surviving observer who helps ensure the orderly transition of power and assists the process of reparative reconstruction.

The function of innate lymphoid cells (ILCs) was originally conceptualized as an "innate" counterpart to Ag-specific Th subsets (Th1, Th2, and Th17) that develop in response to specific pathogens. Indeed, ILC subsets share core developmental programs with their corresponding Th subsets and rely on similar transcriptional regulators for development, maintenance, and cytokine production (1–3). As primarily tissue-resident cells, ILCs are poised as first responders at barrier sites to elaborate cytokines and shape the subsequent adaptive immune response. In this model, they were thought of as an immunologic "Polonius": forward-acting instructors that prime the inflammatory response

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Abbreviations used in this article: AREG, amphiregulin; CGRP, calcitonin gene-related peptide; ENS, enteric nervous system; GI, gastrointestinal; ILC, innate lymphoid cell; LTi, lymphoid tissue inducer; NCR, natural cytotoxicity receptor; NMU, neuromedin U; RORγt, retinoic acid receptor–related orphan receptor γt; TF, transcription factor; VIP, vasoactive intestinal peptide.

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upon pathogen challenge, potentially later resulting in detrimental tissue destruction.

However, there is now increasing appreciation for homeostatic functions for ILCs in maintaining tissue integrity and shaping host responses well after acute perturbation (3). Thus, ILCs have a balanced, advisory role as a homeostatic "Horatio": integrating diverse environmental cues to engage appropriate repair pathways and mitigate excessive tissue injury. In this perspective, we consider how ILC feedback circuits regulate reparative responses to perturbation using basic control theory. We discuss how these tissue circuits are influenced by ILC development and become dysregulated in cases of inflammatory challenge. Finally, we discuss the implications of ILC homeostatic functions in aiding and advancing human health.

CLASSIFICATION AND DEVELOPMENTAL ORIGINS

ILCs are grouped into five major subsets: NK cells, lymphoid tissue inducer (LTi) cells, ILC1, ILC2, and ILC3, based on their expression of lineage-specifying transcription factors (TFs) and their associated signature cytokine production (1, 4). NK cells are mainly circulating cells that express the TF Eomes and perform granzyme-dependent cytotoxic functions (5). In contrast, the noncytotoxic "helper" ILC1, ILC2, and ILC3 subsets are characterized by long-term tissue residence and closely resemble Th1, Th2, and Th17 subtypes of Th cells, respectively, sharing expression of lineage-defining TFs T-bet, GATA-3, and retinoic acid receptor–related orphan receptor γt (RORγt) and associated signature effector cytokines. LTi cells coordinate the formation of secondary lymphoid tissue such as lymph nodes and Peyer's patches in embryonic development, and their postnatal adult function partially overlaps with a $CCR6^+$ ILC3 subset bearing low or absent expression of natural cytotoxicity receptors (NCR^-) that can also direct and maintain tertiary lymphoid tissues such as cryptopatches (4).

In adult bone marrow, innate and adaptive lymphocytes diverge at the common lymphoid precursor stage, after which a committed early ILC precursor arises with multilineage potential for all ILCs (5–7). Among helper ILC subsets, ILC1s express the lineage-specifying TF T-bet and promote type 1 immune responses through production of IFN-γ and TNF family cytokines (5). ILC2s express the TF GATA-3 and coordinate type 2 immune responses through the production of cytokines such as IL-5, IL-9, and IL-13 (8), usually in the context of helminth infection and allergic inflammation (2, 4). ILC3s include multiple subsets of RORγt-dependent innate lymphocytes and are found mainly at mucosal barriers such as gastrointestinal (GI) tract lamina propria. ILC3s serve as the primary steady-state source of IL-22 (9) and also produce IL-17, driving neutrophil recruitment, antimicrobial peptide production, and enhanced barrier function characteristic of type 3 immunity (10, 11).

In this article, we focus on describing homeostatic functions of ILC1, ILC2, and ILC3 subsets. However, we note that NK cells and ILC1s share overlapping surface markers, rely on similar transcriptional networks in development, and exhibit considerable tissue heterogeneity (12). As such, distinguishing between ILC1s and tissue-resident NK cells has been a subject of continued debate because a unique, stably expressed, and reliable ILC1 phenotype relevant to all peripheral tissue sites has yet to be identified (13). Similarly, the functional distinction between ILC3 subtypes, particularly $CCR6^+$ ILC3s that resemble fetal LTi cells and are frequently referred to as LTi-like lymphocytes (14, 15), makes precise delineation between ILC subsets challenging.

ILCs in adult tissues are influenced by the principle of layered ontogeny, in which waves of recruitment into target tissues occur during transiently open developmental windows. These restricted developmental periods are a generalized feature of vertebrate immune cell development, as first described for peritoneal B1 and B2 cells and later shown applicable to most tissue-resident immune cells, including mast cells, tissue macrophages, dendritic epidermal T cells, and γδ T cell–biased lymphoid progenitors (16).

Layered ILC ontogeny has best been characterized in ILC2s, which are broadly distributed in peripheral sites, including lung, GI tract, tonsils, adipose tissue, and skin. Fate mapping approaches have identified successive waves of ILC2 tissue accumulation during mouse development, with the majority appearing in a postnatal wave of expansion that coincides with the upregulation of type 2 cytokine expression (17). ILC2s from fetal liver or adult bone marrow derive from common lymphoid precursors that, at default, have a genomic organization that favors ILC development and subsequently undergoes rearrangement, tethering, and looping of Id2, GATA-3, and RORα elements to become restricted to an ILC2 progenitor pool (18). Although genomic alterations driving differentiation to other ILC subsets are less well characterized, ILC1/NK and ILC3 development appear to follow similar organizing principles, as shown in fate mapping experiments tracing the development of liver-resident NK cells from ILC2 progenitors and in distinct ILC3 subsets (NCR^+ and NCR^-) successively arising in the mouse fetal gut, as recently overviewed (19).

Layered ontogeny studies suggest a model where innate and innate-like lymphocytes expand and disperse in the early postnatal period, become poised for rapid effector function, and exhibit an increased propensity for regulatory, anti-inflammatory phenotypes after encountering self or nonthreatening danger signals (20–22). Analogous to distinct regulatory roles seen in fetus-derived versus monocyte-derived macrophages (23), the homeostatic roles of fetus-derived ILCs residing in tissue niches appear temporally restricted, and later replenishment by ILC progenitors or mature ILC2s from other anatomic compartments, especially after severe inflammatory stress, may not recapitulate perinatally imprinted regulatory functions. For example, after helminth infection, lung tissue–resident "natural" ILC2s exhibited IL-33 responsiveness and a more regulatory phenotype (with higher arginase expression), whereas recruited "inflammatory" ILC2s remained responsive to IL-25

signaling and exhibited shorter-lived, ILC3-like responses in mouse lung (24).

SENSING PERTURBATION AND DANGER SIGNALS

ILCs diverge from the traditional conception of lymphocytes as Ag-specific effector cells of adaptive immunity. For one, they can develop in the absence of the RAGs, RAG1 and RAG2, and express either nonrearranged or minimally rearranged transcripts for TCR chains, suggesting a potential shared origin from IL7R$^+$ lymphoid primed multipotent progenitors that fail to undergo or attempt abortive thymic T-cell selection (16). In contrast to myeloid cells, ILCs rarely rely on pattern recognition through the TLR and NOD-like receptor families (25, 26), instead sensing tissue perturbation through both "canonical" cytokine signals and a bevy of noncanonical (nonpeptide) signals such as neurotransmitters, lipids, dietary metabolites, and other small molecules (2, 3). An overview of this array of sensed ILC environmental inputs is provided in Fig. 1. This sensing can occur directly through cell surface receptors or via chemosensory intermediary cells such as neurons and tuft cells. Traditional cytokine-based stimulation of ILCs via IL receptors, "alarmins," and TNF superfamily members has recently been overviewed elsewhere (2, 3, 16).

Neuromodulators

Most peripheral tissues are innervated by sensory neurons that monitor environmental stress, with their sensory input relayed to ILCs via neurotransmitters and neuropeptides. Examples of neuromodulator responsiveness include adipose tissue ILC2s that express β-adrenergic receptors responsive to sympathetic catecholamines (27) and lung-resident ILC2s expressing the α7 nicotinic acetylcholine receptor responsive to parasympathetic activation (28). Among neuropeptides, products of the glucagon/incretin (e.g., vasoactive intestinal peptide [VIP]), calcitonin (e.g., calcitonin gene-related peptide [CGRP]), and bombesin (e.g., neuromedin U [NMU]) families have all been shown to regulate ILC function in diverse tissue settings, particularly in barrier mucosal tissues such as the lung, the GI tract, and its associated enteric nervous system (ENS) (3).

As select examples, ENS-derived VIP induces IL-5 production from ILC2 in oscillating production cycles to synchronize eosinophil numbers during feeding in one striking example that links innate immunity to circadian rhythm, organismal foraging, and nutrient extraction (29). Similarly, the neuropeptide NMU intrinsically activates gut ILC2s and induces their expansion and activation via NMUR1-Gαq (30, 31), whereas intestinal CGRP restricts IL-13 production, limits an inflammatory KLRG1$^+$ ILC2 pool, and promotes basal IL-5 production (32). Intestinal CCR6$^+$ ILC3s bearing high VIPR2 receptor expression also respond to ENS-derived VIP during food intake (33), which drives IL-22 production and altered localization via homing receptor expression (34). Additionally, pulmonary C-fibers and neuroendocrine cells localized at airway branch points

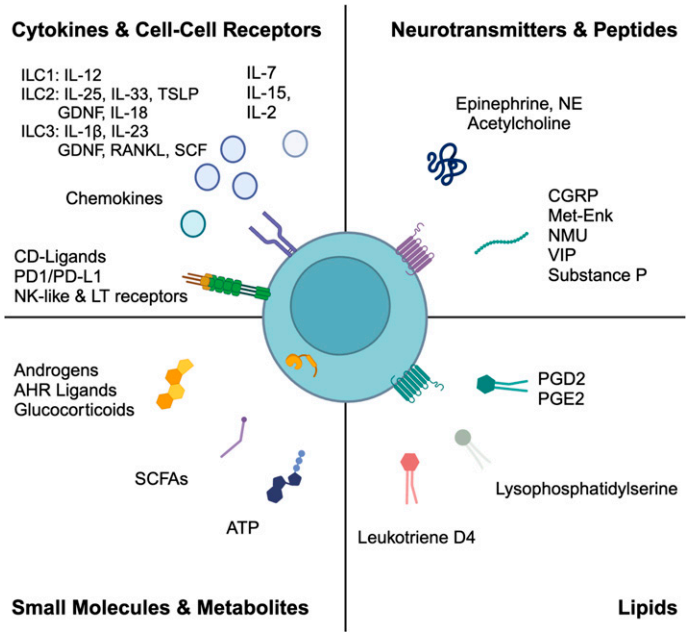

FIGURE 1. Diverse sensory inputs for ILCs.

ILCs respond to external stimuli through the detection of multiple chemosensory cues and second messengers, here divided into four major categories. Similar to adaptive lymphocytes, ILCs are responsive to soluble cytokine signals and *trans* surface receptor engagement in cell–cell ligand–receptor pairs. Additionally, they are linked to the peripheral autonomic nervous system and via neurotransmitters (catecholamines and cholinergic ligands) and neuropeptides of the glucagon/incretin (VIP), calcitonin (CGRP), and bombesin (NMU) families. Lipid signaling mediators include the eicosanoids (leukotrienes and prostaglandins) and tissue damage–associated glycolipids. Finally, multiple metabolites and small molecules, including short-chain fatty acids (SCFAs), ATP, and aryl hydrocarbons (AHRs) also function as local sensory input. Figure created in BioRender.com.

produce CGRP to direct pulmonary ILC2 function, with CGRP acting as a context-dependent negative regulator constraining IL-13 expression and proliferation (35–37).

Lipids, metabolites, and other small molecules

ILCs are also regulated by diverse "innate" (nonpeptide) signals, including lipids (e.g., PGs and leukotrienes) and microbially derived and self-derived small molecules. For example, in a model of liver injury, the extracellular release of ATP from damaged hepatocytes engages P2RX7 receptor-expressing ILC1s to amplify tissue damage and apoptosis downstream of CD155-DNAM1 signaling (38). In the GI tract, PGE_2 signaling sustains intestinal homeostasis in large part through the regulation of EP4 receptor–positive ILC3s, which become stimulated to produce IL-22 (39). Eicosanoid lipid signaling to ILCs can be either pro- or anti-inflammatory, because ILC2s are activated by PGD_2 and leukotriene D_4 but inhibited by PGE_2 and lipoxin A_4 (2). Noneicosanoid lipids also appear to have regulatory roles, as in the case of lysophosphatidylserine release following

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neutrophil apoptosis upon intestinal epithelial injury, which induces IL-22 production in ILC3s via GPR34 (40).

ILC function is also modulated by dietary metabolites, such as vitamin A–derived small molecules signaling through the aryl hydrocarbon receptors and short-chain fatty acids acting on free fatty acid receptors. In the GI tract, aryl hydrocarbon receptor agonists derived upon consumption of cruciferous vegetables promote ILC3 and impair ILC2 function, whereas vitamin D_3 metabolites impair ILC3 activity (41, 42). Similar to eicosanoids, short-chain fatty acids such as acetate, butyrate, and propionate can have pro- and anti-inflammatory effects on ILCs due to differential expression of metabolite-sensing free fatty acid receptors, depending on tissue context (41, 43).

Altogether, ILCs sense and integrate a diverse array of small-molecule and chemical signals released by tissue stress and thus are not reliant solely on cytokine signaling to drive their effector function. The wide range of "innate" (nonpeptide) chemical and small-molecule mediators of ILC function show a striking degree of overlap with nonpeptide ligands that activate innate-like unconventional lymphocytes. These include glycolipids and self-derived carbohydrates stimulating natural IgM-expressing B1 cells, microbiota- and self-derived α-linked glycosylceramides activating NKT cells, and microbiota-derived vitamin B metabolites recognized by MR1-restricted mucosal-associated invariant T cells (44). Unconventional innate-like lymphocytes also share similar developmental signatures to ILCs, such as expression of the TF PLZF (7); have a restricted repertoire of Ig gene rearrangement; and frequently exhibit long-term tissue residence with poised effector function. We propose that further studies rooted in comparative evolutionary analyses and developmental ontogeny of lymphocytes will be essential in deciphering how danger and stress signal recognition arose in ILCs, because similar approaches have been fruitful in understanding TCRα diversity (45) and the animal ancestry of hematopoietic cells (46).

EXAMPLE ILC TISSUE CIRCUITS

Thus far, we have reviewed signaling crosstalk between ILCs and specific epithelial, stromal, and chemosensory cells using linear signaling descriptions. However, we propose that a richer understanding is obtained by invoking control theory (Fig. 2), where tissue circuits are conceptualized as forming a localized, closed-loop negative feedback circuit that maintains organismal homeostasis near an adaptive set point. In diverse cases, ILCs function as the central controller component integrating diverse inputs from surrounding sensors and subsequently relay their signal processing as instructions to output effectors, or actuators, of the circuit (Fig. 2A). Example circuits have best been described for ILC2, but similar principles appear applicable to ILC1, ILC3, and innate-like unconventional lymphocytes that share developmental pathways and local regulation by noncanonical innate danger signals.

The tuft cell–ILC2 circuit is particularly illustrative as a case study. Tuft cells are rare, endoderm-derived chemosensory epithelial cells sharing similarity to type 2 taste receptor cells that detect sweet, bitter, and umami taste in the oropharynx (47). Expression of GPR91 allows tuft cells to detect succinate generated by *Tritrichomonas* protists, an intestinal commensal of wild mice (48). Tuft cells respond by releasing IL-25, which stimulates lamina propria ILC2s to produce type 2 cytokines (IL-5 and IL-13) and growth factors such as amphiregulin (AREG) (49, 50) upon pathobiont colonization. IL-13 acts directly on crypt transit-amplifying intestinal stem cells to slow their transit time and alter their migration as they ascend the intestinal villi (51, 52). Increased dwell time at the base of intestinal crypts biases stem cell differentiation toward secretory cell fates (e.g., goblet and tuft cells) at the expense of transitioning to absorptive enterocytes (49, 53). This tuft cell–mediated biasing underlies much of the type 2 immune "weep and sweep" response that clears helminth parasites and adjusts tissue cell composition during protist colonization (Fig. 2B).

Similar tissue circuits where ILC2s function as central controllers to direct homeostatic and reparative type 2 immunity have been uncovered in models of influenza infection of the pulmonary tract (54) and dextran sulfate sodium–induced colitis of the lower GI tract (55). In these cases, the ILC2 tissue repair response was more strongly linked to production of AREG, a growth factor that binds epidermal growth factor receptors on epithelial cells to promote cell proliferation. This was substantiated with the recent use of Nmur-Cre–driven specific deletion of AREG in ILC2s, which led to disrupted colonic homeostasis and delayed clearance after *Trichuris* infection (56).

ILC3s also maintain homeostasis in the GI tract during enteric pathogen infection or upon perturbation of the intestinal microbiota (2, 3) by chemical- or radiation-induced tissue damage (Fig 2C) via a similarly organized tissue circuit. Multiple ILC3 subsets produce IL-22, which signals to GI tract epithelial cells to induce a diverse barrier immunity program that includes the upregulation of antimicrobial Reg and defensin proteins, heightened production and altered glycosylation of mucins, and increased tight junction permeability (9, 57–59). Akin to ILC2-derived IL-13 action on intestinal stem cells, ILC3-derived IL-22 modulates stem cell identity and differentiation by preserving $Lgr5^+$ intestinal stem cells after injury (58) to sustain epithelial regenerative capacity, with supplemental reinforcement by production of ILC3-derived reparative growth factors such as GM-CSF (60) and heparin binding epidermal growth factor (61, 62), somewhat analogous to the production of ILC2-derived AREG.

In a final example, ILC2s function as a central actuator in allergic atopic behavior such as itch and food avoidance as part of an adaptive homeostatic response that limits physical proximity and future exposure to potentially tissue-destructive, noxious stimuli (Fig 2D). In a tape stripping–based model of itch, damaged keratinocytes release systemic IL-33 to synergize with tuft cell–derived IL-25 to drive ILC2 and Th2 cell activation in the GI tract. This induces local release of IL-13 and IL-4 that expands small intestinal mast cells, which function as allergic effectors in adjusting intestinal permeability and anaphylactic

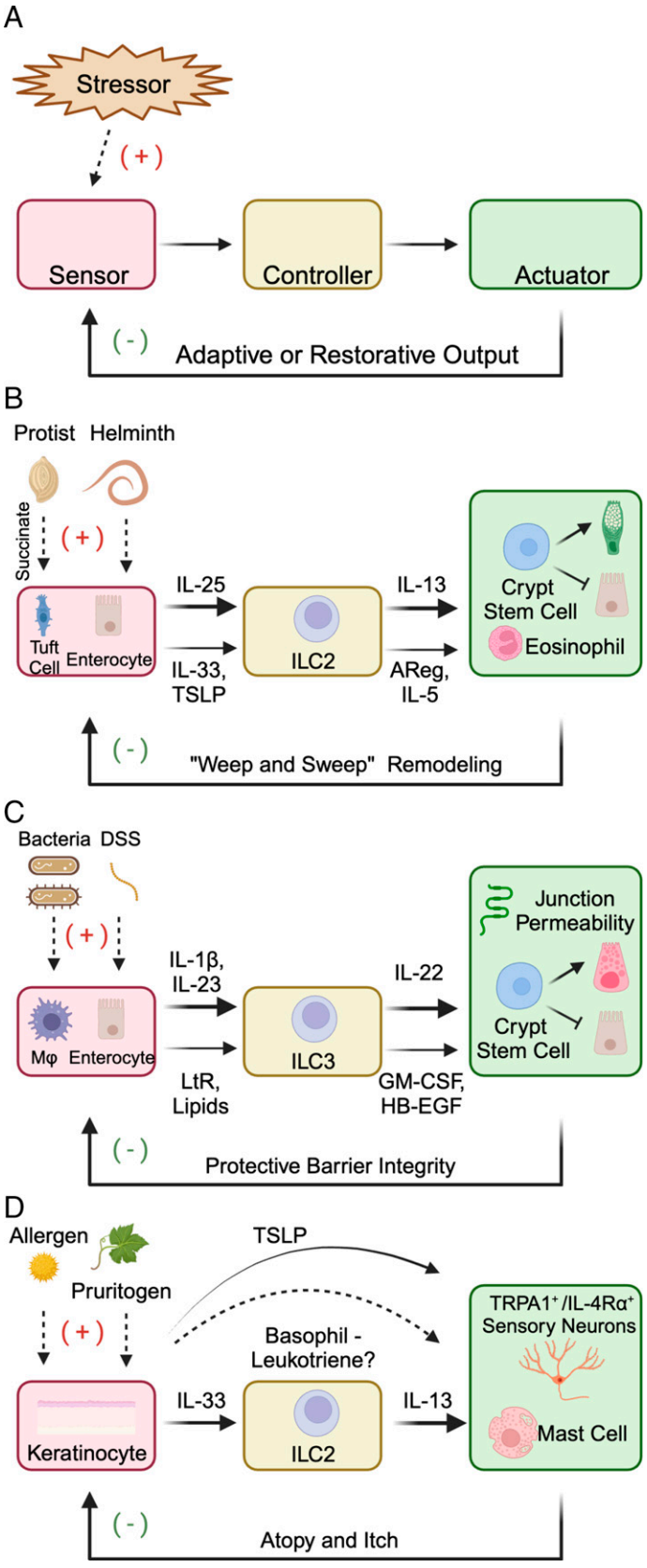

FIGURE 2. ILCs function as controllers in local closed-loop feedback circuits to maintain homeostasis and adapt to external stressors. (**A**) Using control theory principles, local tissue circuits are constructed with a framework of ILCs serving as central controllers, where they receive environmental inputs from surrounding sensor cells that become perturbed by external stressors (disturbance). ILCs integrate sensory cues to then relay appropriate executive instructions to effector outputs (actuators), typically through cytokine production, to generate an appropriate homeostatic or reparative response to accommodate the external disturbance and restore tissue function back to the adaptive set point. (**B**) ILC2s function as controllers in the GI tract during protist colonization or helminth invasion to engage a "weep and sweep" protective tissue response. (**C**) ILC3s function as controllers in the GI tract during disrupted active bacterial infection or chemical and environmental injury to engage a protective barrier immunity tissue response. (**D**) ILC2s function as controllers in the skin and the GI tract during pruritogen or allergen exposure to engage a protective itch and atopic response. Figure created in BioRender.com. DSS, dextran sulfate sodium; HB-EGF, heparin binding epidermal growth factor; TSLP, thymic stromal lymphopoietin.

responses in food allergy (63). Separately, in a model of atopic dermatitis–like conditioned skin inflammation, the mast cell–histamine axis was found dispensable for itch, instead requiring a basophil–leukotriene axis (64), suggesting that although ILCs broadly function as central controllers, they can direct distinct actuator components depending on the tissue context.

Although current studies have supported this model of ILCs functioning as the central "Horatio" controller, multiple knowledge gaps still exist in our circuit framework. In particular, the actuator-driven resolution of tissue responses to injury or stress, as well as the subsequent restoration of homeostasis, is often incomplete and unpredictable, with varied and unsynchronized timescales. In some cases, such as pathogenic fibrosis resulting after severe viral infection, actuators do not appropriately dampen down their response even after the removal of the inciting stimuli, resulting in a pathogenic tissue response and the development of interstitial lung disease. Future studies of ILC-centered tissue circuits should focus on understanding actuator output back to (attempted) homeostasis, with attention paid to temporal resolution, adjustment of immune response types, and possible effector variation linked to diverse environmental inputs. This will enable a more fruitful assessment of homeostatic roles of ILCs as they engage tissue remodeling through the modulation of stem cell differentiation, biasing of developmental fates and recruitment of specialized repair cell types. Ultimately, understanding ILC-driven actuator outputs in greater detail will allow their subsequent modulation and redirection, with the eventual mitigation of maladaptive tissue responses such as in chronic fibrosis, the metabolic syndrome, and allergic anaphylaxis.

EFFECTOR PLASTICITY AND MIXED INFLAMMATION

One challenging aspect of interpreting ILC studies lies in an increasing recognition of effector plasticity, or a spectrum of

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phenotypes similar to that observed in Th cell subsets (65). Mature ILCs, especially in inflammatory settings, take on mixed or interchangeable properties of multiple subsets at once rather than adhere to discrete ILC1, ILC2, and ILC3 paradigms. For instance, T-bet is upregulated in CCR6$^-$ ILC3s during *Salmonella* (14) and *Clostridioides difficile* infection (66), endowing them with ILC1-like functions. Pulmonary ILC2s can take on ILC1 properties by downregulating GATA-3 to produce IFN-γ during viral infection and after cigarette smoke exposure (67). Mouse epidermis-resident ILCs at baseline exhibit simultaneous ILC2 and ILC3 properties, including coexpression of GATA-3 and RORγt (68), which was also described in human dermal ILC2s cocultured with *Candida* (69). This so-called pathogenic effector state of skin-resident ILCs was later shown to be due to a preexisting dense developmental continuum that spans the ILC2 and ILC3 transcriptional landscape even at steady state (70).

Although effector plasticity can make disentangling immunopathology challenging, it also highlights the importance of appreciating layered ontogeny and systemic ILC-poiesis in uncovering ILC function. Like the earlier example of inflammatory ILC2s in the lung, developmental timing may explain the distinct functional properties of NCR$^-$ and NCR$^+$ gut ILC3s, which exhibit contrasting patterns of IL-17 and IL-22 production and reflect their differing origins in fetal gut versus postnatal bone marrow (19). Indeed, many of the differences found between mouse and human, including increased circulating ILC2 progenitor cells and more abundant adaptive T-cell residence in human tissues at birth (20, 21), appear to reflect chronologic differences in murine versus human fetal immune development. We argue that a fuller understanding of ILC effector plasticity and mixed inflammation will be crucial to adequately characterizing organismal responses to physiologic stimuli, because many environmental stressors do not fall neatly into type 1, 2, or 3 paradigms.

CLINICAL IMPLICATIONS AND CONCLUSIONS

Altogether, a cross-disciplinary body of work supports a model of ILCs functioning as deeply embedded controllers in complex tissue circuits, integrating environmental signals received from feedback sensors to engage downstream restorative cellular actuators (Fig. 2). The continued study of ILC tissue circuits in this framework will be crucial in understanding the sequelae and consequences of immune modulation in diverse clinical populations, not only during acute infection but also, for example, in oncology patients maintained on long-term immune checkpoint inhibitor therapy (71–73); rheumatologic patients treated with long-term biologic therapies targeting ILC-relevant cytokines such as IL-4Ra, IL-5, and IL12/23; and individuals receiving therapies directed against small signaling mediators that impact tissue lymphocyte function, such as migraine patients treated with CGRP antagonists.

Rather than serve only as a frontline marshal to mediate combative measures against pathogens, akin to the Polonius role of aiding Claudius, ILCs report and deliver on tissue dramas of variegated form and function, ranging from traumatic injury, noxious chemical stimuli, pathobiont colonization, and nutrient availability. In this regard, they resemble the versatile, all-purpose adviser and sounding board that Horatio represents for Prince Hamlet. In so doing, ILCs engage in oft underrecognized roles on the frontlines of repair and restoration, like the gravediggers who toil in obscurity in the final act of Hamlet—truly no more ancient gentlemen but the gardeners, ditchers, and gravemakers that hold up Adam's profession.

DISCLOSURES

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REFERENCES

1. Spits, H., D. Artis, M. Colonna, A. Diefenbach, J. P. Di Santo, G. Eberl, S. Koyasu, R. M. Locksley, A. N. J. McKenzie, R. E. Mebius, et al. 2013. Innate lymphoid cells—a proposal for uniform nomenclature. *Nat. Rev. Immunol.* 13: 145–149.
2. Klose, C. S. N., and D. Artis. 2020. Innate lymphoid cells control signaling circuits to regulate tissue-specific immunity. *Cell Res.* 30: 475–491.
3. Xiong, L., S. L. Nutt, and C. Seillet. 2022. Innate lymphoid cells: more than just immune cells. *Front. Immunol.* 13: 1033904.
4. Vivier, E., D. Artis, M. Colonna, A. Diefenbach, J. P. Di Santo, G. Eberl, S. Koyasu, R. M. Locksley, A. N. J. McKenzie, R. E. Mebius, et al. 2018. Innate lymphoid cells: 10 years on. *Cell* 174: 1054–1066.
5. Klose, C. S. N., M. Flach, L. Möhle, L. Rogell, T. Hoyler, K. Ebert, C. Fabiunke, D. Pfeifer, V. Sexl, D. Fonseca-Pereira, et al. 2014. Differentiation of type 1 ILCs from a common progenitor to all helper-like innate lymphoid cell lineages. *Cell* 157: 340–356.
6. Yang, Q., F. Li, C. Harly, S. Xing, L. Ye, X. Xia, H. Wang, X. Wang, S. Yu, X. Zhou, et al. 2015. TCF-1 upregulation identifies early innate lymphoid progenitors in the bone marrow. *Nat. Immunol.* 16: 1044–1050.
7. Constantinides, M. G., B. D. McDonald, P. A. Verhoef, and A. Bendelac. 2014. A committed precursor to innate lymphoid cells. *Nature* 508: 397–401.
8. Hoyler, T., C. S. N. Klose, A. Souabni, A. Turqueti-Neves, D. Pfeifer, E. L. Rawlins, D. Voehringer, M. Busslinger, and A. Diefenbach. 2012. The transcription factor GATA-3 controls cell fate and maintenance of type 2 innate lymphoid cells. *Immunity* 37: 634–648.
9. Sonnenberg, G. F., L. A. Fouser, and D. Artis. 2011. Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. *Nat. Immunol.* 12: 383–390.
10. Mills, K. H. G. 2023. IL-17 and IL-17-producing cells in protection versus pathology. *Nat. Rev. Immunol.* 23: 38–54.
11. Eyerich, K., V. Dimartino, and A. Cavani. 2017. IL-17 and IL-22 in immunity: driving protection and pathology. *Eur. J. Immunol.* 47: 607–614.
12. Seillet, C., L. Brossay, and E. Vivier. 2021. Natural killers or ILC1s? That is the question. *Curr. Opin. Immunol.* 68: 48–53.
13. Nabekura, T., and A. Shibuya. 2021. Type 1 innate lymphoid cells: soldiers at the front line of immunity. *Biomed. J.* 44: 115–122.
14. Klose, C. S. N., E. A. Kiss, V. Schwierzeck, K. Ebert, T. Hoyler, Y. d'Hargues, N. Göppert, A. L. Croxford, A. Waisman, Y. Tanriver, and A. Diefenbach. 2013. A T-bet gradient controls the fate and function of CCR6-RORγt$^+$ innate lymphoid cells. *Nature* 494: 261–265.
15. Sciumé, G., K. Hirahara, H. Takahashi, A. Laurence, A. V. Villarino, K. L. Singleton, S. P. Spencer, C. Wilhelm, A. C. Poholek, G. Vahedi,

et al. 2012. Distinct requirements for T-bet in gut innate lymphoid cells. *J. Exp. Med.* 209: 2331–2338.
16. Molofsky, A. B., and R. M. Locksley. 2023. The ins and outs of innate and adaptive type 2 immunity. *Immunity* 56: 704–722.
17. Schneider, C., J. Lee, S. Koga, R. R. Ricardo-Gonzalez, J. C. Nussbaum, L. K. Smith, S. A. Villeda, H.-E. Liang, and R. M. Locksley. 2019. Tissue-resident group 2 innate lymphoid cells differentiate by layered ontogeny and in situ perinatal priming. *Immunity* 50: 1425–1438.e5.
18. Michieletto, M. F., J. J. Tello-Cajiao, W. K. Mowel, A. Chandra, S. Yoon, L. Joannas, M. L. Clark, M. T. Jimenez, J. M. Wright, P. Lundgren, et al. 2023. Multiscale 3D genome organization underlies ILC2 ontogenesis and allergic airway inflammation. *Nat. Immunol.* 24: 42–54.
19. Kogame, T., G. Egawa, T. Nomura, and K. Kabashima. 2022. Waves of layered immunity over innate lymphoid cells. *Front. Immunol.* 13: 957711.
20. Lim, A. I., Y. Li, S. Lopez-Lastra, R. Stadhouders, F. Paul, A. Casrouge, N. Serafini, A. Puel, J. Bustamante, L. Surace, et al. 2017. Systemic human ILC precursors provide a substrate for tissue ILC differentiation. *Cell* 168: 1086–1100.e10.
21. Spits, H., and J. Mjösberg. 2022. Heterogeneity of type 2 innate lymphoid cells. *Nat. Rev. Immunol.* 22: 701–712.
22. Constantinides, M. G., and Y. Belkaid. 2021. Early-life imprinting of unconventional T cells and tissue homeostasis. *Science* 374: eabf0095.
23. Park, M. D., A. Silvin, F. Ginhoux, and M. Merad. 2022. Macrophages in health and disease. *Cell* 185: 4259–4279.
24. Huang, Y., L. Guo, J. Qiu, X. Chen, J. Hu-Li, U. Siebenlist, P. R. Williamson, J. F. Urban, Jr., and W. E. Paul. 2015. IL-25-responsive, lineage-negative KLRG1(hi) cells are multipotential 'inflammatory' type 2 innate lymphoid cells. *Nat. Immunol.* 16: 161–169.
25. She, L., G. D. Barrera, L. Yan, H. H. Alanazi, E. G. Brooks, P. H. Dube, Y. Sun, H. Zan, D. P. Chupp, N. Zhang, et al. 2021. STING activation in alveolar macrophages and group 2 innate lymphoid cells suppresses IL-33-driven type 2 immunopathology. *JCI Insight* 6: e143509.
26. Okuzumi, S., J. Miyata, H. Kabata, T. Mochimaru, S. Kagawa, K. Masaki, M. Irie, H. Morita, and K. Fukunaga. 2021. TLR7 agonist suppresses group 2 innate lymphoid cell-mediated inflammation via IL-27-producing interstitial macrophages. *Am. J. Respir. Cell Mol. Biol.* 65: 309–318.
27. Moriyama, S., J. R. Brestoff, A.-L. Flamar, J. B. Moeller, C. S. N. Klose, L. C. Rankin, N. A. Yudanin, L. A. Monticelli, G. G. Putzel, H.-R. Rodewald, and D. Artis. 2018. β_2-Adrenergic receptor-mediated negative regulation of group 2 innate lymphoid cell responses. *Science* 359: 1056–1061.
28. Yuan, F., L. Jiang, Q. Li, L. Sokulsky, Y. Wanyan, L. Wang, X. Liu, L. Zhou, H. L. Tay, G. Zhang, et al. 2021. A selective α7 nicotinic acetylcholine receptor agonist, PNU-282987, attenuates ILC2s activation and *Alternaria*-induced airway inflammation. *Front. Immunol.* 11: 598165.
29. Nussbaum, J. C., S. J. Van Dyken, J. von Moltke, L. E. Cheng, A. Mohapatra, A. B. Molofsky, E. E. Thornton, M. F. Krummel, A. Chawla, H.-E. Liang, and R. M. Locksley. 2013. Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature* 502: 245–248.
30. Klose, C. S. N., T. Mahlakõiv, J. B. Moeller, L. C. Rankin, A.-L. Flamar, H. Kabata, L. A. Monticelli, S. Moriyama, G. G. Putzel, N. Rakhilin, et al. 2017. The neuropeptide neuromedin U stimulates innate lymphoid cells and type 2 inflammation. *Nature* 549: 282–286.
31. Cardoso, V., J. Chesné, H. Ribeiro, B. García-Cassani, T. Carvalho, T. Bouchery, K. Shah, N. L. Barbosa-Morais, N. Harris, and H. Veiga-Fernandes. 2017. Neuronal regulation of type 2 innate lymphoid cells via neuromedin U. *Nature* 549: 277–281.
32. Xu, H., J. Ding, C. B. M. Porter, A. Wallrapp, M. Tabaka, S. Ma, S. Fu, X. Guo, S. J. Riesenfeld, C. Su, et al. 2019. Transcriptional atlas of intestinal immune cells reveals that neuropeptide α-CGRP modulates group 2 innate lymphoid cell responses. *Immunity* 51: 696–708.e9.
33. Talbot, J., P. Hahn, L. Kroehling, H. Nguyen, D. Li, and D. R. Littman. 2020. Feeding-dependent VIP neuron-ILC3 circuit regulates the intestinal barrier. *Nature* 579: 575–580.
34. Yu, H. B., H. Yang, J. M. Allaire, C. Ma, F. A. Graef, A. Mortha, Q. Liang, E. S. Bosman, G. S. Reid, J. A. Waschek, et al. 2021. Vasoactive intestinal peptide promotes host defense against enteric pathogens by modulating the recruitment of group 3 innate lymphoid cells. *Proc. Natl. Acad. Sci. USA* 118: e2106634118.
35. Sui, P., D. L. Wiesner, J. Xu, Y. Zhang, J. Lee, S. Van Dyken, A. Lashua, C. Yu, B. S. Klein, R. M. Locksley, et al. 2018. Pulmonary neuroendocrine cells amplify allergic asthma responses. *Science* 360: eaan8546.
36. Wallrapp, A., P. R. Burkett, S. J. Riesenfeld, S.-J. Kim, E. Christian, R.-E. E. Abdulnour, P. I. Thakore, A. Schnell, C. Lambden, R. H. Herbst, et al. 2019. Calcitonin gene-related peptide negatively regulates alarmin-driven type 2 innate lymphoid cell responses. *Immunity* 51: 709–723.e6.
37. Nagashima, H., T. Mahlakõiv, H.-Y. Shih, F. P. Davis, F. Meylan, Y. Huang, O. J. Harrison, C. Yao, Y. Mikami, J. F. Urban, Jr., et al. 2019. Neuropeptide CGRP limits group 2 innate lymphoid cell responses and constrains type 2 inflammation. *Immunity* 51: 682–695.e6.
38. Nabekura, T., L. Riggan, A. D. Hildreth, T. E. O'Sullivan, and A. Shibuya. 2020. Type 1 innate lymphoid cells protect mice from acute liver injury via interferon-γ secretion for upregulating Bcl-xL expression in hepatocytes. *Immunity* 52: 96–108.e9.
39. Duffin, R., R. A. O'Connor, S. Crittenden, T. Forster, C. Yu, X. Zheng, D. Smyth, C. T. Robb, F. Rossi, C. Skouras, et al. 2016. Prostaglandin E_2 constrains systemic inflammation through an innate lymphoid cell-IL-22 axis. *Science* 351: 1333–1338.
40. Wang, X., J. Cai, B. Lin, M. Ma, Y. Tao, Y. Zhou, L. Bai, W. Jiang, and R. Zhou. 2021. GPR34-mediated sensing of lysophosphatidylserine released by apoptotic neutrophils activates type 3 innate lymphoid cells to mediate tissue repair. *Immunity* 54: 1123–1136.e8.
41. Li, S., J. W. Bostick, J. Ye, J. Qiu, B. Zhang, J. F. Urban, Jr., D. Avram, and L. Zhou. 2018. Aryl hydrocarbon receptor signaling cell intrinsically inhibits intestinal group 2 innate lymphoid cell function. *Immunity* 49: 915–928.e5.
42. Spencer, S. P., C. Wilhelm, Q. Yang, J. A. Hall, N. Bouladoux, A. Boyd, T. B. Nutman, J. F. Urban, Jr., J. Wang, T. R. Ramalingam, et al. 2014. Adaptation of innate lymphoid cells to a micronutrient deficiency promotes type 2 barrier immunity. *Science* 343: 432–437.
43. Yang, W., T. Yu, X. Huang, A. J. Bilotta, L. Xu, Y. Lu, J. Sun, F. Pan, J. Zhou, W. Zhang, et al. 2020. Intestinal microbiota-derived short-chain fatty acids regulation of immune cell IL-22 production and gut immunity. *Nat. Commun.* 11: 4457.
44. Van Kaer, L., J. L. Postoak, W. Song, and L. Wu. 2022. Innate and innate-like effector lymphocytes in health and disease. *J. Immunol.* 209: 199–207.
45. Giorgetti, O. B., C. P. O'Meara, M. Schorpp, and T. Boehm. 2023. Origin and evolutionary malleability of T cell receptor α diversity. *Nature* 619: 193–200.
46. Nagahata, Y., K. Masuda, Y. Nishimura, T. Ikawa, S. Kawaoka, T. Kitawaki, Y. Nannya, S. Ogawa, H. Suga, Y. Satou, et al. 2022. Tracing the evolutionary history of blood cells to the unicellular ancestor of animals. *Blood* 140: 2611–2625.
47. Schneider, C., C. E. O'Leary, and R. M. Locksley. 2019. Regulation of immune responses by tuft cells. *Nat. Rev. Immunol.* 19: 584–593.
48. Howitt, M. R., S. Lavoie, M. Michaud, A. M. Blum, S. V. Tran, J. V. Weinstock, C. A. Gallini, K. Redding, R. F. Margolskee, L. C. Osborne, et al. 2016. Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science* 351: 1329–1333.
49. von Moltke, J., M. Ji, H.-E. Liang, and R. M. Locksley. 2016. Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. *Nature* 529: 221–225.
50. Schneider, C., C. E. O'Leary, J. von Moltke, H.-E. Liang, Q. Y. Ang, P. J. Turnbaugh, S. Radhakrishnan, M. Pellizzon, A. Ma, and R. M. Locksley. 2018. A metabolite-triggered tuft cell-ILC2 circuit drives small intestinal remodeling. *Cell* 174: 271–284.e14.

51. Sanman, L. E., I. W. Chen, J. M. Bieber, V. Steri, C. Trentesaux, B. Hann, O. D. Klein, L. F. Wu, and S. J. Altschuler. 2021. Transit-amplifying cells coordinate changes in intestinal epithelial cell-type composition. *Dev. Cell* 56: 356–365.e9.
52. Lindholm, H. T., N. Parmar, C. Drurey, M. Campillo Poveda, P. M. Vornewald, J. Ostrop, A. Díez-Sanchez, R. M. Maizels, and M. J. Oudhoff. 2022. BMP signaling in the intestinal epithelium drives a critical feedback loop to restrain IL-13-driven tuft cell hyperplasia. *Sci. Immunol.* 7: eabl6543.
53. Gerbe, F., E. Sidot, D. J. Smyth, M. Ohmoto, I. Matsumoto, V. Dardalhon, P. Cesses, L. Garnier, M. Pouzolles, B. Brulin, et al. 2016. Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. *Nature* 529: 226–230.
54. Monticelli, L. A., G. F. Sonnenberg, M. C. Abt, T. Alenghat, C. G. K. Ziegler, T. A. Doering, J. M. Angelosanto, B. J. Laidlaw, C. Y. Yang, T. Sathaliyawala, et al. 2011. Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nat. Immunol.* 12: 1045–1054.
55. Monticelli, L. A., L. C. Osborne, M. Noti, S. V. Tran, D. M. W. Zaiss, and D. Artis. 2015. IL-33 promotes an innate immune pathway of intestinal tissue protection dependent on amphiregulin-EGFR interactions. *Proc. Natl. Acad. Sci. USA* 112: 10762–10767.
56. Tsou, A. M., H. Yano, C. N. Parkhurst, T. Mahlakõiv, C. Chu, W. Zhang, Z. He, K. J. Jarick, C. Zhong, G. G. Putzel, et al.; JRI IBD Live Cell Bank Consortium. 2022. Neuropeptide regulation of non-redundant ILC2 responses at barrier surfaces. *Nature* 611: 787–793.
57. Gronke, K., P. P. Hernández, J. Zimmermann, C. S. N. Klose, M. Kofoed-Branzk, F. Guendel, M. Witkowski, C. Tizian, L. Amann, F. Schumacher, et al. 2019. Interleukin-22 protects intestinal stem cells against genotoxic stress. *Nature* 566: 249–253.
58. Lindemans, C. A., M. Calafiore, A. M. Mertelsmann, M. H. O'Connor, J. A. Dudakov, R. R. Jenq, E. Velardi, L. F. Young, O. M. Smith, G. Lawrence, et al. 2015. Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration. *Nature* 528: 560–564.
59. Hanash, A. M., J. A. Dudakov, G. Hua, M. H. O'Connor, L. F. Young, N. V. Singer, M. L. West, R. R. Jenq, A. M. Holland, L. W. Kappel, et al. 2012. Interleukin-22 protects intestinal stem cells from immune-mediated tissue damage and regulates sensitivity to graft versus host disease. *Immunity* 37: 339–350.
60. Pearson, C., E. E. Thornton, B. McKenzie, A.-L. Schaupp, N. Huskens, T. Griseri, N. West, S. Tung, B. P. Seddon, H. H. Uhlig, and F. Powrie. 2016. ILC3 GM-CSF production and mobilisation orchestrate acute intestinal inflammation. *eLife* 5: e10066.
61. Zhou, L., W. Zhou, A. M. Joseph, C. Chu, G. G. Putzel, B. Fang, F. Teng, M. Lyu, H. Yano, K. I. Andreasson, et al. 2022. Group 3 innate lymphoid cells produce the growth factor HB-EGF to protect the intestine from TNF-mediated inflammation. *Nat. Immunol.* 23: 251–261.
62. Mortha, A., A. Chudnovskiy, D. Hashimoto, M. Bogunovic, S. P. Spencer, Y. Belkaid, and M. Merad. 2014. Microbiota-dependent crosstalk between macrophages and ILC3 promotes intestinal homeostasis. *Science* 343: 1249288.
63. Leyva-Castillo, J.-M., C. Galand, C. Kam, O. Burton, M. Gurish, M. A. Musser, J. D. Goldsmith, E. Hait, S. Nurko, F. Brombacher, et al. 2019. Mechanical skin injury promotes food anaphylaxis by driving intestinal mast cell expansion. *Immunity* 50: 1262–1275.e4.
64. Wang, F., A. M. Trier, F. Li, S. Kim, Z. Chen, J. N. Chai, M. R. Mack, S. A. Morrison, J. D. Hamilton, J. Baek, et al. 2021. A basophil-neuronal axis promotes itch. *Cell* 184: 422–440.e17.
65. Murphy, K. M., and B. Stockinger. 2010. Effector T cell plasticity: flexibility in the face of changing circumstances. *Nat. Immunol.* 11: 674–680.
66. Abt, M. C., B. B. Lewis, S. Caballero, H. Xiong, R. A. Carter, B. Sušac, L. Ling, I. Leiner, and E. G. Pamer. 2015. Innate immune defenses mediated by two ILC subsets are critical for protection against acute *Clostridium difficile* infection. *Cell Host Microbe* 18: 27–37.
67. Silver, J. S., J. Kearley, A. M. Copenhaver, C. Sanden, M. Mori, L. Yu, G. H. Pritchard, A. A. Berlin, C. A. Hunter, R. Bowler, et al. 2016. Inflammatory triggers associated with exacerbations of COPD orchestrate plasticity of group 2 innate lymphoid cells in the lungs. [Published erratum appears in 2016 *Nat. Immunol.* 17: 1005.] *Nat. Immunol.* 17: 626–635.
68. Kobayashi, T., B. Voisin, D. Y. Kim, E. A. Kennedy, J.-H. Jo, H.-Y. Shih, A. Truong, T. Doebel, K. Sakamoto, C.-Y. Cui, et al. 2019. Homeostatic control of sebaceous glands by innate lymphoid cells regulates commensal bacteria equilibrium. *Cell* 176: 982–997.e16.
69. Bernink, J. H., Y. Ohne, M. B. M. Teunissen, J. Wang, J. Wu, L. Krabbendam, C. Guntermann, R. Volckmann, J. Koster, S. van Tol, et al. 2019. c-Kit-positive ILC2s exhibit an ILC3-like signature that may contribute to IL-17-mediated pathologies. [Published erratum appears in 2020 *Nat. Immunol.* 21: 101.] *Nat. Immunol.* 20: 992–1003.
70. Bielecki, P., S. J. Riesenfeld, J.-C. Hütter, E. Torlai Triglia, M. S. Kowalczyk, R. R. Ricardo-Gonzalez, M. Lian, M. C. Amezcua Vesely, L. Kroehling, H. Xu, et al. 2021. Skin-resident innate lymphoid cells converge on a pathogenic effector state. *Nature* 592: 128–132.
71. Jacquelot, N., C. Seillet, M. Wang, A. Pizzolla, Y. Liao, S. Hediyeh-Zadeh, S. Grisaru-Tal, C. Louis, Q. Huang, J. Schreuder, et al. 2021. Blockade of the co-inhibitory molecule PD-1 unleashes ILC2-dependent antitumor immunity in melanoma. *Nat. Immunol.* 22: 851–864.
72. Moral, J. A., J. Leung, L. A. Rojas, J. Ruan, J. Zhao, Z. Sethna, A. Ramnarain, B. Gasmi, M. Gururajan, D. Redmond, et al. 2020. ILC2s amplify PD-1 blockade by activating tissue-specific cancer immunity. *Nature* 579: 130–135.
73. Jou, E., N. Rodriguez-Rodriguez, A. F. Ferreira, H. E. Jolin, P. A. Clark, K. Sawmynaden, M. Ko, J. E. Murphy, J. Mannion, C. Ward, et al. 2022. An innate IL-25-ILC2-MDSC axis creates a cancer-permissive microenvironment for *Apc* mutation-driven intestinal tumorigenesis. *Sci. Immunol.* 7: eabn0175.